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Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and *in vitro* toxicity

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ABSTRACT

Mainstream smoke (MS) from experimental kretek cigarettes with three ingredient mixes at low (typical use level) and high (2.5 or 3 times that level) inclusion rates was compared to a control kretek cigarette of identical construction (cloves and humectants), but without the addition of ingredients. 350 ingredients, commonly used in various combinations and in a limited number in a given brand in the manufacture of marketed kretek cigarettes were assessed. The MS composition of the kretek cigarettes was characterized by a comprehensive set of analytes (55 smoke constituents). Furthermore, the smoke was assessed *in vitro* for its cytotoxicity in the Neutral Red Uptake assay (particle phase and gas/vapor phase separately) in mouse embryo BALB/c 3T3 cells, and for mutagenicity/genotoxicity in the *Salmonella typhimurium* reverse mutation assay and the mammalian cell mouse lymphoma TK assay in L5178Y cells, the latter with and without metabolic activation. There were some statistically significant differences in the yield of smoke constituents (increases as well as decreases, nearly all of them less than ±20%) as a result of the addition of the ingredient mixes. However, the addition of the three different mixes of ingredients to the experimental kreteks did not change the *in vitro* cytotoxicity and mutagenicity/genotoxicity of the smoke, when compared to the control kretek cigarette.

1. Introduction

This publication is part of a series summarizing the *in vitro* and *in vivo* toxicological assessment of kretek cigarettes. Smoke composition and biological activity of mainstream smoke (MS) from marketed and experimental kretek cigarettes were evaluated on a comparative basis in smoke chemistry analyses, and *in vitro* and *in vivo* toxicity studies. The studies were designed to cover three main topics: (1) characterization of kreteks and comparison relative to American-blended cigarettes, (2) impact of blend type and cloves, and (3) impact of ingredients used in kretek cigarettes. Further in depth information of this assessment is described in the lead publication (Roemer, 2014b).

Kretek cigarettes contain clove buds in addition to tobacco in the filler (up to 40%) that gives them their unique taste and smell. In addition to the clove buds they also contain flavor ingredients according to the smoking preferences of the different consumer groups. Other ingredients are used to facilitate tobacco processing and to preserve tobacco moisture (LaVoie et al., 1986). Historically, many tobacco farmers applied a substance mixture of undisclosed, secret composition, the sauce, already to the tobacco before delivery to the cigarette manufacturers (Polzin et al., 2007). The sauce consisted mainly of flavors and substances that release flavors upon combustion. Today, at least for the main kretek cigarette manufacturers, the sauce has been replaced by the addition of a mix of defined flavoring and moistening substances to the tobacco in the factory with full disclosure of the single ingredients by the vendors to allow a review whether a single ingredient might increase the inherent toxicity of the smoke at the level used.

More than 400 of the ingredients used in various combinations in American-blended cigarettes have been tested for their effects on smoke chemistry and toxicological activity (Gaworski et al., 1999; Heck et al., 2002; Roemer et al., 2002; Rustemeier et al., 2002; Vanscheeuwijck et al., 2002; Baker and Bishop, 2004; Baker et al., 2004a,b; Roemer et al., 2004; Renne et al., 2006; Gaworski et al., 2011; Purkis et al., 2011). Many of the ingredients

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Table 1
Cigarette specifications.

Specification	Cigarette type						
	Control	Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
Cigarette weight (g)	1.44	1.41	1.42	1.43	1.43	1.46	1.40
Blend weight (g)	1.22	1.19	1.20	1.21	1.21	1.24	1.18
Clove weight (g)	0.38	0.37	0.37	0.38	0.38	0.38	0.37
Eugenol (mg)	3.64	4.21	3.88	4.60	4.40	3.56	3.49
Humectants weight (mg)	21.00	20.50	20.60	20.80	20.80	21.30	20.30
Ingredients weight (mg)	0.00	142.00	383.00	145.00	413.00	149.00	425.00
Cigarette length (mm)	89.90	89.90	90.00	89.80	90.10	90.20	90.10
Circumference (mm)	24.80	24.80	24.80	24.90	24.80	24.80	24.80
Paper porosity (C.U.)	57.80	57.40	58.90	57.40	57.20	56.60	57.30

Remarks: blend is comprising tobacco and cloves, eugenol refers to content in tobacco.

used in American-blended cigarettes are used also in kreteks. However, as the combination of cloves and ingredients may lead to effects which are not observable in American-blended cigarettes, these ingredients together with those used exclusively in kretek cigarettes were investigated. The ingredients were incorporated into the tobacco blend in three different mixes (termed Mix A, B, and C).

Comprehensive analyses were made of smoke chemistry, with a target of 55 smoke constituents. This list of analytes is based on recommendations from two groups of public health experts: the International Agency on Cancer Research and the US Consumer Product Safety Commission (IARC, 1985, US-CPSC, 1993). Nearly identical lists have been applied at other laboratories investigating cigarette smoke (Bombick et al., 1998; Gaworski et al., 1999; Chen and Moldoveanu, 2003; Stavanja et al., 2006; Potts et al., 2010). Cigarettes were also tested for their *in vitro* cytotoxicity using the Neutral Red Uptake (NRU) assay described by Borenfreund and Puerner (1985). Two mutagenicity/genotoxicity assays were included as they were recommended in the guidelines of the International Conference on Harmonization developed for pharmaceuticals (US-DHHS, 2008). These assays were the conventional and widely used *Salmonella* Reverse Mutation Assay in 5 bacterial strains developed by Ames et al. (1973) and the Mouse Lymphoma Assay (MLA) using L5178Y cells (Clive et al., 1972). It has been shown previously that this mammalian cell genotoxicity assay can be reliably used to assess and compare the mutagenicity of TPM (Schramke et al., 2006). At least the NRU assay and the Ames Assay have become a quasi-standard in cigarette smoke toxicology (Baker et al., 2004a; Stavanja et al., 2006; Potts et al., 2010; Dempsey et al., 2011; Gaworski et al., 2011; Roemer et al., 2012).

Part 7 of this series of publications, reports on the results of the inhalation toxicity of mainstream smoke of the kretek cigarettes with the addition of the ingredient mixes A, B, and C (Schramke et al., 2014).

2. Materials and methods

2.1. General

Detailed descriptions regarding the chemical analyses, toxicological assays and statistical procedures can be found in the first of this series of publications (Roemer et al., 2014b).

For logistic reasons the NRU assay and the MLA were performed with kretek ingredient Mixes A and B in separate experiments to Mix C.

2.2. Cigarettes

A total of 350 ingredients, commonly used in various combinations and in a limited number in a given brand in the manufacture of marketed kretek cigarettes, were assigned to three different ingredient mixes, i.e., Mix A, Mix B, and Mix C (see Appendix, Table A). The ingredients were introduced in the respective mix at a low and a high target level. The addition levels to these test cigarettes were the typical use level and either 2.5 (licorice extract and invert sugar) or three times the use level. The mixes were added to experimental cigarettes containing, beside the Indonesian tobacco, 31% cut cloves and 1.7% humectants (propylene glycol, glycerin, and D-sorbitol) as filler, similar to the Kretek-R reported earlier (Piadé et al., 2014). Further details on the cigarettes can be found in Table 1. As the cigarettes were manufactured to a constant weight, the ingredients partly replaced the filler. They were added to the filler at that point in the cigarette production process (casing and after-cut) where they are normally added in the industrial fabrication process, some ingredients were therefore added to more than one mix. There were differences in target/addition concentrations of the individual ingredients concentrations in tobacco and those after the manufacturing process. These differences were considered to represent normal manufacturing losses due to evaporation or chemical interaction with tobacco components.

The list of tested ingredients, their regulatory status and the target concentrations in each mix is given in the Appendix in Table A. Each ingredient was of food grade quality. A cigarette of identical construction (cloves and humectants) but without the addition of ingredients, served as control cigarette. Control and test cigarettes were produced by PT Hanjaya Mandala Sampoerna Tbk., Surabaya, Indonesia. The American-blended reference cigarette 2R4F was used as internal reference cigarette. The reference cigarette 2R4F was purchased from the University of Kentucky, Kentucky Tobacco Research and Development Center (Davis and Vaught, 1990).

2.3. Smoke generation

The control and test cigarettes were conditioned for at least 48 h under a fume hood in their original, sealed packs at 22 ± 2 °C and relative humidity of 60 ± 5%. They were then unpacked and transferred in sealed storage boxes for transportation to the smoking machine. The 2R4F cigarettes were conditioned according to ISO standard 3402 (ISO, 1999).The cigarettes were smoked on a 20-port Borgwaldt smoking machine RM20H under ISO smoking conditions (ISO, 1991a). In short, puff volume, puff duration, and puff frequency for the ISO smoking conditions were 35 ml, 2 s, and 1/min. Cigarettes were smoked to a final butt length of 35 mm.

2.4. Smoke chemistry

In addition to the ISO analytes, TPM, nicotine, carbon monoxide (CO), and water (ISO, 1991b, 2000, 2007), further analytes were selected based on two source documents: a proposal that specifically focused on smoke chemistry testing from the US Consumer

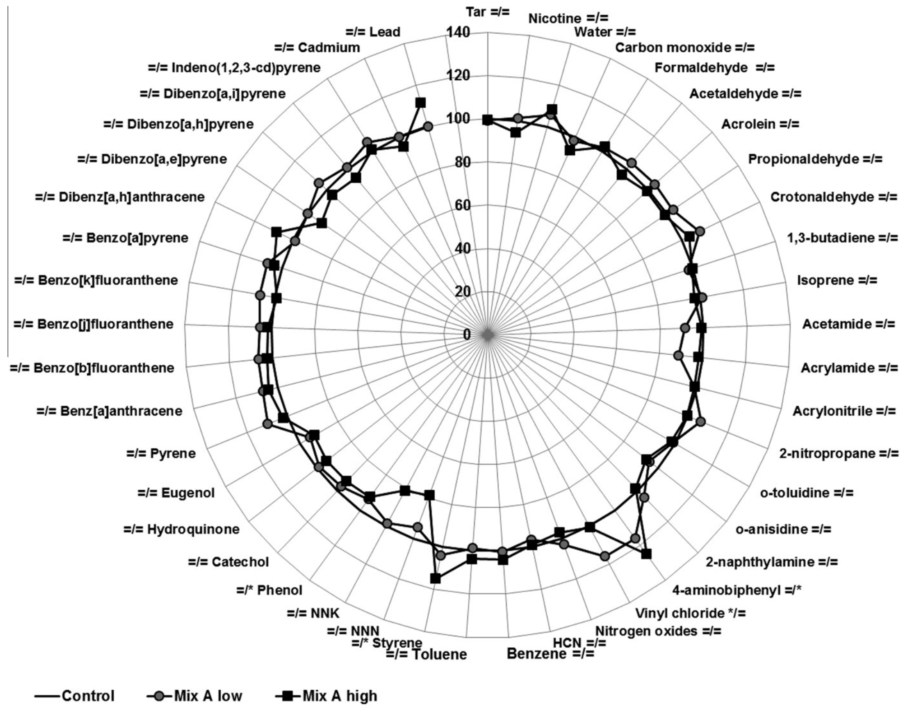

Fig. 1. Smoke chemistry, cigarettes with inclusion of Mix A at a low and high level relative to control cigarette (%), on a per mg TPM basis. Remarks: =, difference statistically not significant; *, statistically different; first symbol represents comparison of Mix at low level to control; second symbol comparison of Mix at high level to control.

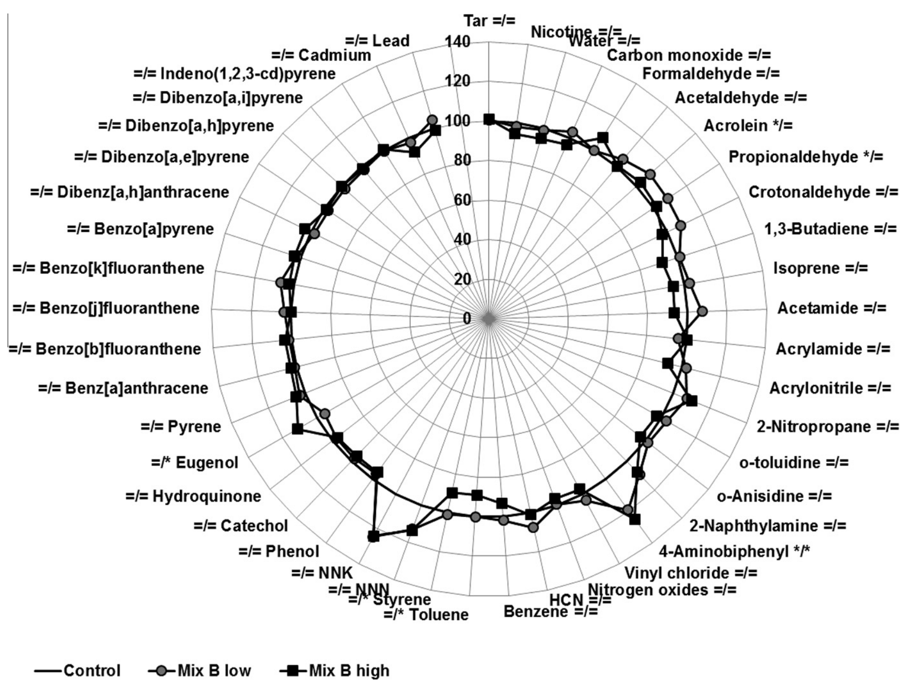

Fig. 2. Smoke chemistry, cigarettes with inclusion of Mix B at a low and high level relative to control cigarette (%), on a per mg TPM basis. Remarks: =, difference statistically not significant; *, statistically different; first symbol represents comparison of Mix at low level to control; second symbol comparison of Mix at high level to control.

Product Safety Commission (US-CPSC, 1993) and a monograph from the International Agency for Research on Cancer (IARC, 1985). Analytes were quantified according to established methodology as previously described (Roemer et al., 2004).

2.5. In vitro cytotoxicity

Cytotoxicity of TPM and the water soluble constituents of the gas/vapor phase (GVP) of MS was assessed with the Neutral Red Uptake (NRU) assay (Borenfreund and Puerner, 1985, 1987) with mouse embryo BALB/c 3T3 cells as previously described (Roemer et al., 2009).

2.6. In vitro bacterial mutagenicity

Mutagenicity of TPM was assessed in the plate incorporation *Salmonella* Reverse Mutation Assay (Maron and Ames, 1983)

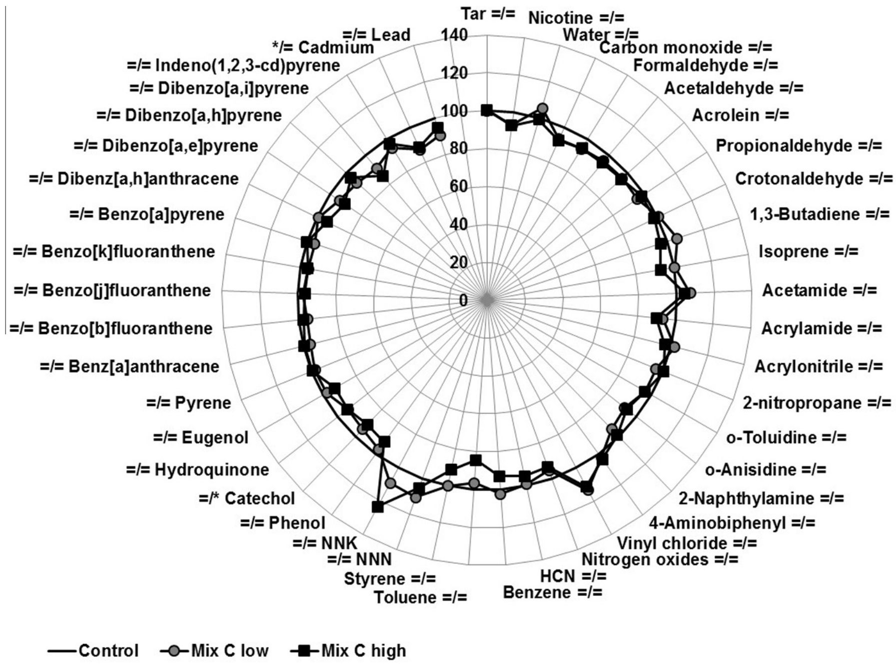

Fig. 3. Smoke chemistry, cigarettes with inclusion of Mix C at a low and high level relative to control cigarette (%), on a per mg TPM basis. Remarks: =, difference statistically not significant; *, statistically different; first symbol represents comparison of Mix at low level to control; second symbol comparison of Mix at high level to control.

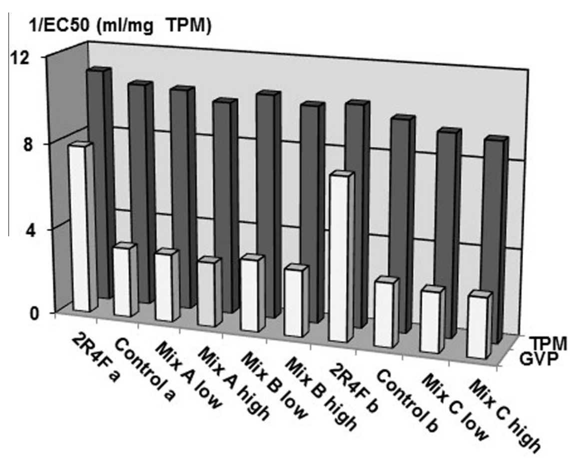

Fig. 4. *In vitro* cytotoxicity on a per mg TPM basis of American-blended reference cigarette 2R4F and experimental cigarettes containing no (control) or inclusions of kretek ingredient mixes A, B, and C. Data are expressed as mean ($n = 3$) of the $1/EC_{50}$ values.

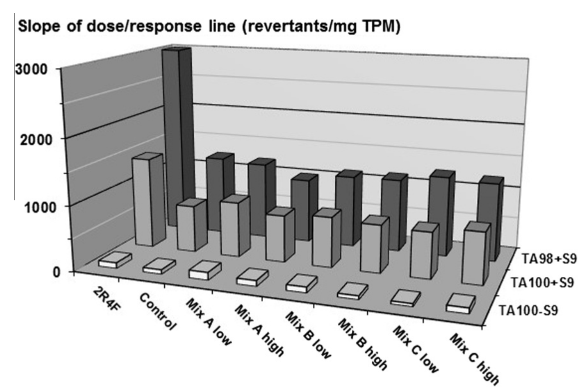

Fig. 5. *In vitro* bacterial mutagenicity on a per mg TPM basis of American-blended reference cigarette 2R4F and experimental cigarettes containing no (control) or inclusions of kretek ingredient mixes A, B, and C. Mutagenicity results are expressed as mean ($n = 2$, with 3 plates per batch) of the revertants/mg TPM values.

according to the respective OECD guideline 471 (OECD, 1997a) as previously described (Roemer et al., 2012).

Determinations were performed in the presence and in the absence of a metabolic activation system.

2.7. In vitro mammalian cell genotoxicity

Genotoxicity of TPM was assessed in the Mouse Lymphoma TK Assay (MLA) developed by Clive et al. (1972). It was performed according to OECD guideline 476 (OECD, 1997b) in the microtiter plate version (Cole et al., 1986) with L5178Y cells with and without metabolic activation as described in detail by Schramke et al. (2006).

2.8. Statistics

Results obtained for the test cigarette groups were compared to the control cigarette group with standard statistical tools. The reference cigarette 2R4F was only used as an internal reference to monitor consistency with historical data of the laboratory and was not included in the statistical evaluation.

3. Results

3.1. Smoke chemistry

The following smoke constituents were below the limit of quantitation (LOQ) in smoke from control and/or test kretek cigarettes, and consequently they will not be discussed further: N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-propylamine(NPRA),

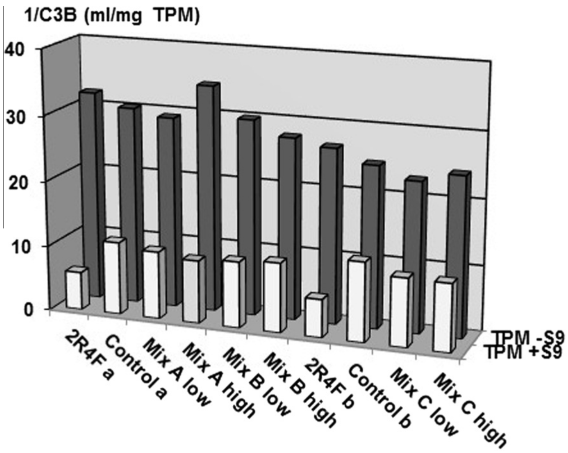

Fig. 6. *In vitro* genotoxicity in mouse lymphoma cells on a per mg TPM basis of American-blended reference cigarette 2R4F and experimental cigarettes containing no (control) or inclusions of kretek ingredient mixes A, B, and C. Mutagenicity results are given as $1/C_{3B}$ values calculated over the two TPM batches tested. Remarks: 2R4F a and Control a refers to Mix A and B, 2R4F b and Control b refers to Mix C as groups tested parallel.

N-nitrosodi-n-butylamine(NBUA), N-nitrosopyrrolidine (NPY), N-nitrosopiperidine (NPI), dibenz[a,h]anthracene, dibenz[a,h]pyrene, dibenz[a,i]pyrene, dibenzo[a,l]pyrene, 5-methylchrysene, nickel, arsenic, and chromium.

Results on a per cigarette basis are presented in the in the Appendix in Table B. Results expressed on a per mg total particulate matter (TPM) basis relative to the control cigarette are presented in Figs. 1–3. There were a few sporadic significant differences between control and test cigarettes, but nearly all of them were smaller than ±20% and there was generally no relationship between smoke constituent yield and added ingredient levels. As the TPM deliveries of the test and control cigarettes were similar, the small differences between the cigarettes remain the same as on a per cigarette basis when expressed in percentage terms.

3.2. In vitro cytotoxicity

Acrolein as positive control substance (data not shown) as well as TPM and GVP of the reference cigarette 2R4F produced cytotoxicity results consistent with historical data from the laboratory.

Cytotoxicity results on a per cigarette basis are given in the Appendix in Table C. There were no significant differences between the mean $1/EC_{50}$ values for Mixes A, B and C, compared with the appropriate control cigarette data, for both the TPM and the GVP.

When compared on a per mg TPM basis, according to the nearly identical TPM yield of the cigarettes, there were also no significant differences between control and test cigarettes for both the TPM and GVP fractions (Fig. 4). Cytotoxicity of TPM from the reference cigarette 2R4F was approximately the same as that from the experimental cigarettes (control and test), but the cytotoxicity of the GVP was approximately twice as high.

3.3. In vitro bacterial mutagenicity

Strain- and S9-specific positive control substances (data not shown) as well as TPM of the reference cigarette 2R4F produced mutagenicity results consistent with historical data from the laboratory.

The tester strains TA102 and TA1535, with and without S9, were unresponsive to the treatment with TPM and are not discussed further.

Mutagenicity results of TPM from the control and test cigarettes on a per cigarette basis are given in the Appendix in Table C. There were no significant differences between the mutagenicity of TPM from the test cigarettes containing either Mix A, B or C when compared to that of the control cigarette.

On a per mg TPM basis, mutagenicity results for TPM of the test cigarettes containing Mix A, B or C were very similar to those of the control cigarette, for both low and high inclusions. There were no cases of significant differences from the control cigarette. The most responsive strain for all cigarettes tested was TA98 with S9 (approximately 1100 revertants per mg TPM for the experimental cigarettes, and 3000 revertants per mg TPM for the reference cigarette 2R4F); the least responsive strain with discriminative ability was TA100 without S9 (around 70 revertants per mg TPM for each cigarette type). See Fig. 5 for the mutagenicity results observed in the tester strains TA98 and TA100 with S9, and TA100 without S9 for the TPM of the tested cigarettes.

3.4. In vitro mammalian cell genotoxicity

Methyl methanesulfonate and benzo[*a*]pyrene as positive control substances (data not shown) as well as the TPM of the reference cigarette 2R4F produced mutagenicity results consistent with historical data from the laboratory.

Genotoxicity results on a per cigarette basis are given in the Appendix in Table C. There were no significant differences between the genotoxicity of TPM from the test cigarettes containing either Mix A, B or C when compared to that of the control cigarette.

On a per mg TPM basis, results for TPM of the test cigarettes containing Mix A, B or C were very similar to those of the control cigarette, for both low and high inclusions as well as with and without metabolic activation. There were no cases of significant differences from the control cigarette. Without metabolic activation, mutagenicity data for the reference cigarette 2R4F were approximately the same as for the experimental cigarettes. Values with S9 were approximately two times lower for the reference cigarette 2R4F (Fig. 6).

4. Discussion

In this study three mixes of ingredients typically used in the manufacture of kretek cigarettes were tested. Addition of ingredients to kretek cigarettes at levels up to 3-fold of their use level did not discernibly alter the smoke chemistry profile of the selected major toxic constituents of smoke. The lack of differences in smoke composition of the experimental cigarettes with and without ingredients is mirrored by a lack of differences in the *in vitro* toxicity.

This result confirms the findings observed in a study on ingredients used in American-blended cigarettes (Carmines, 2002), as approximately 260 of the ingredients tested here had been tested there without any evidence that the addition of ingredients to the tobacco might increase the inherent toxicity of cigarette smoke.

There is an increasing trend to report cigarette emission data per mg nicotine, as, e.g., suggested by authorative bodys, e.g., World Health Organization (WHO, 2009), but also by individual researchers (Rickert et al., 2007, 2011; Ashley et al., 2008). This might be useful, e.g., when comparing different brand available on the market. However, in the conduct of the toxicological assessment of ingredient, this may be misleading (DIN, 2014). Often, the experimental cigarettes, used in this context, contain a rather high concentration (normally not found in market cigarettes) of ingredients that do not emit any nicotine into the smoke. Thus, a normalization to nicotine would lead to higher emission data for the test cigarettes with the addition of ingredients compared to control cigarette without ingredients. In our studies, reported here, the test cigarettes did not differ more than 6% compared to the control. As such, a normalization to nicotine has no considerable influence on the comparisons and conclusions, compared to our normalization to TPM.

The authors have chosen the ISO smoking regimen in this study for two reasons. First, it has been shown that more intense smoking regimens, e.g., required by Health Canada (1999), are less efficient in detecting possible differences in the yield of toxicants when comparing different cigarette types Carchman (Roemer and Carchman, 2011). Second, more intense smoking regimens than the ISO obviously overestimate the smoke uptake by human smokers of cigarettes with higher tar yields, e.g., around 10 mg ISO tar. For kretek cigarettes with a yield of up to 50 mg ISO tar the application of intense smoking regimens would lead to estimated tar yields of far more than 100 mg per cigarette. These values have obviously no relationship to the actual intake of a smoker.

Although not within the scope of the studies presented here, it might be interesting to compare the *in vitro* response of the American-blended reference cigarette 2R4F with that of the experimental kreteks. As the particulate phase showed the same cytotoxicity, the GVP of the experimental kretek cigarette smoke is distinctly lower in activity. Also the mutagenic activity of the particulate phase of the kretek smoke is only approximately one half and less in the most responsive *Salmonella* tester strains. This confirms the data obtained in other *in vitro* experiments within this set of studies (Piadé et al., 2014). In the mouse lymphoma assay, however, the mutagenic activity of TPM from the experimental kreteks in the presence of metabolic activation, was double that of the reference cigarette 2R4F. This is in agreement with the response toward pure eugenol obtained in this series of studies (Roemer et al., 2014a) or observed by other researchers (Tennant et al., 1987; Myhr and Caspary, 1991). It is possible that this is a false positive, as this assay is known for a rather high rate of false positives (Lorge et al., 2007; Reeve et al., 2012). In other *in vitro* and *in vivo* genotoxicity assays, eugenol shows mixed results, although most of them are negative (EFSA, 2009). Eugenol did not show carcinogenic activity in rats and mice, when administered orally (FDA, 1983; Miller et al., 1983). It has been argued that eugenol is positive in some genotoxicity tests but not carcinogenic, because it only causes one type of mutation due to one type of DNA adduct (however, for cancer, several mutations are required). This one adduct may stimulate repair mechanisms that have antigenotoxic effects. This could explain the equivocal results in geneotoxicity testing (Tisserand and Young, 2014).

Taken together, the results suggest that adding ingredients to kretek cigarettes at typical use levels does not adversely alter the smoke chemistry or *in vitro* biological effects normally associated with exposure to mainstream kretek cigarette smoke.

Conflict of interest statement

All authors are or were Philip Morris International (PMI) employees. The work reported here was funded by PMI R&D. This work was supported in Part by Philip Morris USA, Inc. until the spin-off of Philip Morris International, Inc. by Altria Group, Inc. on March 26, 2008.

Acknowledgments

The authors are grateful to the staff at Hanjaya Mandala Sampoerna Tbk Indonesia, who designed and manufactured the experimental cigarettes according to demanding specifications. Equally, they thank the involved staffs Philip Morris Research Laboratories, Cologne, Germany, for their excellent technical performance.

Appendix.

See Tables A–C.

Table A
Listing of ingredients tested.

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
1,8-Cineole	470-82-6	2465	182	1234	172.515	1	3				
1-Octen-3-ol	3391-86-4	2805	72	1152	172.515					1	3
1-Pentanol	71-41-0	2056	514	88	172.515					1	3
2-Methylpyrazine	109-08-0	3309	2270	761		1	3			1	3
2,3,5,6-Tetramethylpyrazine	1124-11-4	3237	2210	780		1	3			1	3
2,3,5-Trimethylpyrazine	14667-55-1	3244	735	774		1	3			1	3
2,3-Diethylpyrazine	15707-24-1	3136	534	771		1	3			1	3
2,5-Dimethylpyrazine	123-32-0	3272	2210	766		1	3			1	3
2,6-Dimethoxyphenol	91-10-1	3137	2233	721		1	3			1	3
2-Ethoxy-3,5-(or 6)-methylpyrazine	65504-94-1	3569	11921	793		1	3				
2-Ethyl-3,(5 or 6)-dimethylpyrazine	13925-07-0	3149	727	775		1	3			1	3
2-Ethyl-3-methylpyrazine	15707-23-0	3155	548	768						1	3
2-Heptanone	110-43-0	2544	136	283	172.515	1	3			1	3
2-Methoxy-4-methylphenol	93-51-6	2671	175	715	172.515	1	3			1	3
2-Methyl-2-pentenoic acid	3142-72-1	5289		1210		1	3				
2-Methylbutyric acid	116-53-0	2695	2002	255	175.515			3	9	36	108
2-Pentanone	107-87-9	2842	754	279	175.515			1	3		
2-Propanethiol	75-33-2	3897	11565	510				1	3		
3,7-Dimethyl-1,3,6-octatriene	13877-91-3	3539	11741	1338	172.515					1	3
3-Carbomethoxy pyridine	93-60-7	3709		1320						23	69
3-Ethylpyridine	536-78-7	3394	11386	1315		1	3			1	3
3-Methyl pentanoic acid	105-43-1	3437	10149	262		1	3			1	3
3-Methyl-2-butanol	598-75-4	3703		300		10	30	1	3		
3-Methyl-butyraldehyde	590-86-3	2692	94	258	172.515	1	3			1	3
3-Propylidenephthalide	17369-59-4	2952	494	1168	172.515					1	3
4-(Para-hydroxyphenyl)-2-butanone	5471-51-2	2588	755	728	172.515	2	6			1	3

(*continued on next page*)

Table A (*continued*)

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
4,5-Dimethyl-3-hydroxy-2(5H)-furanone	28664-35-9	3634		243						1	3
4-Carvomenthenol	562-74-3	2248	2229	439	172.515	2	6			1	3
4-Ethylguaiacol	2785-89-9	2436	176	716	172.515	1	3			3	9
4-Methyl-5-thiazoleethanol	137-00-8	3204	11621	1031		3	9			5	15
4-Methylacetophenone	122-00-9	2677	156	807	172.515	7	21			1	3
5-Methylquinoxaline	13708-12-8	3203	2271	798						1	3
6-Methyl-5-hepten-2-one	110-93-0	2707	149	1120	172.515			1	3	1	3
Acetanisole	100-06-1	2005	570	810	172.515			1	3	1	3
Acetic acid	64-19-7	2006	2	81	184.1005	22	66			23	69
Acetoin	513-86-0	2008	749	405	182.60	2	6			1	3
Acetophenone	98-86-2	2009	138	806	172.515			5	15	9	27
Acetyl propionyl	600-14-6	2841	2039	410	172.515	1	3			1	3
Acetylpyrazine	22047-25-2	3126	2286	784		1	3			1	3
A–D-glucose	50-99-7									1	3
Allspice oil (pimenta berry)	8006-77-7	2018	335		182.20			188	564	1	3
Allyl hexanoate	123-68-2	2032	2181	3	172.515			4	12	51	153
Alpha iso-methyl ionone	127-51-5	2714	169	404	172.515			1	3		
Alpha-ionone	127-41-3	2594	141	388	172.515	3	9			5	15
Alpha-methyl benzyl acetate	93-92-5	2684	573	801	172.515	1	3				
Alpha-pinene	80-56-8	2902	2113	1329	172.515			1	3	13	39
Alpha-terpineol	98-55-5	3045	62	366	172.515					1	3
Amyl acetate	628-63-7		211					1	3		
Amyl formate	638-49-3	2068	497	119	172.515	22	66	2	6		
Amyl hexanoate	540-07-8	2074	315	163	1752.515					3	9
Amyris balsamifera extract	8015-65-4		33		172.510			1	3		
Angelica root oil	8015-64-3	2088	56		182.20			3	9	1	3
Anise oil extract	8007-70-3	2094						46	138		
Anisyl acetate	104-21-2	2098	209	873	172.515	1	3	2	6		
Anisyl alcohol	105-13-5	2099	66	2001	172.515					3	9
Anisyl alcohol	1331-81-3					7	21				
Apple juice concentrate	85251-63-4					1	3				
Ascorbic acid	50-81-7	2109	27		182.3013	1	3			1	3
Basil oil	8015-73-4	2119	308		182.20					2	6
Benzaldehyde	100-52-7	2127	101	22	182.60	93	279			2	6
Benzoic acid	65-85-0	2131	21	850	184.1021	1	3			18	54
Benzoin, resinoid	9000-05-9	2133	439		182.20	32	96	1	3		
Benzoin, resinoid	9000-72-0									2	6
Benzyl acetate	140-11-4	2135	204	23	172.515			1	3	4	12
Benzyl alcohol	100-51-6	2137	58	25	172.515	522	1566			68	204
Benzyl benzoate	120-51-4	2138	262	24	172.515	171	513			2	6
Benzyl butyrate	103-37-7	2140	277	843	172.515					1	3
Benzyl cinnamate	103-41-3	2142	331	670	172.515	7	21			1	3
Benzyl isovalerate	103-38-8	2152	453	845	172.515					2	6
Benzyl propionate	122-63-4	2150	413	842	172.515					1	3
Benzyl salicylate	118-58-1	2151	436	904	172.515	1	3				
Bergamot oil	8007-75-8	2153	137		182.20			20	60		
Beta-caryophyllene	87-44-5	2252	2118	1324	172.515			124	372	2	6
Beta-damascenone	23696-85-7	3420	11197	387				1	3	1	3
Trans-beta-damascone	23726-91-2	3243				5	15				
Cis-beta-damascone	23726-92-3		2340	384						5	15
Beta-ionone	14901-07-6	2595	142	389	172.515	1	3			1	3
Beta-pinene	127-91-3	2903	2144	1330	172.515	10	30				
Bisabolene	495-62-5	3331	10979	1336						2	6
Bornyl acetate	76-49-3	2159	2157	1387	172.515			1	3		
Buchu leaves oil	68650-46-4	2169	85		172.510					2	6
Butaraldehyde	123-72-8	2219	91	86	172515	1	3			1	3
Butyl alcohol	71-36-3	2178	52	85	172.515			8	24	3	9
Butyl butyrate	109-21-7	2186	268	151	172.515			20	60	1	3
Butyl butyryllactate	7492-70-8	2190	2107	935	172.515			1	3		
Butyl hexanoate	626-82-4	2201	313	162	172.515			1	3	1	3
Butyl isovalerate	109-19-3	2218	444	198	172.515					2	6
Butyric acid	107-92-6	2221	5	87	182.60	52	156			8	24
Camphene	79-92-5	2229	2227	1323	172.515					2	6
Caramel color	8028-89-5	2235			182.1235			76	228	53	159
Caraway oil	8000-42-8	2238	112		182.20			1	3		
Cardamom seed oil	8000-66-6	2241	180		182.20			1	3	1	3
Carob bean extract	84961-45-5	2243	120		182.20	1	3			24	72
Cassia bark extract	84961-46-6	2290	133		182.20					102	306
Cassia oil	8007-80-5	2258	131		182.20	2	6			2	6
Cedarwood oil	8000-27-9		252					1	3		
Celery seed oil	8015-90-5	2271	52		182.20					3	9

Table A (*continued*)

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
Chamomile flower, roman, extract	8015-92-7	2275	48		182.20			1	3	1	3
Cherry juice concentrate	89997-53-5					80	240				
Chicory extract	68650-43-1	2280	127		182.20					25	75
Cinnamaldehyde	104-55-2	2286	102	656	182.60	306	918	6	18		
Cinnamic acid	621-82-9	2288	22	657	172.515	4	12			1	3
Cinnamon bark extract	977038-60-0	2290	133		182.20	459	1377				
Cinnamon bark oil	8015-91-6	2292	133		182.20			49	147	1	3
Cinnamyl acetate	103-54-8	2293	208	650	172.515			2	6	3	9
Cinnamyl alcohol	104-54-1	2294	65	647	172.515	1	3	2	6		
Cinnamyl cinnamate	122-69-0	2298	332	673	172.515					1	3
Cis-3-hexen-1-yl acetate	3681-71-8	3171	644	134		1	3			1	3
Cis-3-hexenol	928-96-1	2563	750	315	172.515	2	6			1	3
Cis-3-hexenyl lactate	61931-81-5	3690	10681	934		1	3				
Citral	5392-40-5	2303	109	1225	182.60			1	3	8	24
Citric acid monohydrate	5949-29-1			218				5	15	6	18
Citric acid	77-92-9	2306	20	218	184.1033	6	18			5	15
Citronella oil	106-22-9							17	51	1	3
Citronellyl acetate	150-84-5	2311	202	57	172.515			1	3		
Clary oil	8016-63-5	2321	415		182.20					4	12
Clary sage oil	84775-83-7	2321	415		182.20			1	3		
Clove bud extract oleoresin						695	2085				
Clove bud fluid extract								1	3		
Clove oil	8000-34-8	2325	188		184.1257			441	1323	1	3
Cocoa and cocoa products	977075-45-8									4	12
Cocoa extract	84649-99-0					25	75			37	111
Cocoa powder	95009-22-6									335	1005
Cocoa shell extract	8002-31-1							1	3	207	621
Coffee bean oil	8001-67-0		148							172	516
Coffee extract	68916-18-7							21	63		
Coffee extract	84650-00-0				182.20			1	3	577	1731
Cognac oil, green	8016-21-5	2331			182.50			12	36	3	9
Commiphora myrrha extract	8016-37-3	2766	150		172.510			1	3		
Copaiba oil	8013-97-6							1	3	1	3
Coriander oil	8008-52-4	2334	154		182.20			4	12	1	3
Costus root oil	8023-88-9	2336	53		172.510			1	3		
Cubeb oil	8007-87-2	2339	345		172.510			3	9		
Cumin oil	8014-13-9	2343	161		182.20			1	3	1	3
Cyclohexyl acetate	622-45-7	2349	217	1093	172.515			1	3		
Methyl-cyclopentenolone anhydre	765-70-8	2700	758	418	172.515			6	18		
Davana oil	8016-03-3	2359	69	-	172.510			1	3	1	3
Decanal	112-31-2	2362	98	104	182.60			1	3		
Decanoic acid	334-48-5	2364	11	105	172.860	1	3			1	3
Delta-decalactone	705-86-2	2361	621	232	172.515	7	21			1	3
Delta-dodecalactone	713-95-1	2401	624	236	172.515	1	3				
Delta-octalactone	698-76-0	3214	2195	228						1	3
Delta-undecalatone	710-04-3	3294	688	234						2	6
Diacetin	25395-31-7									2	6
Diacetyl	431-03-8	2370	752	408		1	3			1	3
Diethyl malonate	105-53-3	2375	2106	614	172.515	1	3	1	3		
Diethyl sebacate	110-40-7	2376	623	624	172.515			1	3		
Dihydrocarvyl acetate	20777-49-5	2380	2064	379	172.515					3	9
Dimethyl benzyl carbinyl butyrate	10094-34-5	2394	2084	1656	172.515					3	9
D-Limonene	5989-27-5	2633	491	1326	182.60			1	3		
DL-Menthone	89-80-5									4	12
Ethyl 2-methylbutyrate	7452-79-1	2443	265	206	172.515	2	6			1	3
Ethyl 2-octenoate	2351-90-8							1	3		
Ethyl 3-hydroxybutyrate	5405-41-4	3428	10596	594		1	3				
Ethyl 3-hydroxyhexanoate	2305-25-1	3545	11764	601		1	3				
Ethyl acetate	141-78-6	2414	191	27	182.60	11	33	355	1065		
Ethyl acetoacetate	141-97-9	2415	240	595	172.515	7	21			1	3
Ethyl benzoate	93-89-0	2422	261	852	172.515	1	3			1	3
Ethyl butyrate	105-54-4	2427	264	29	182.60			2730	8190	9	27
Ethyl cinnamate	103-36-6	2430	323	659	172.515	2	6			1	3
Ethyl decanoate	110-38-3	2432	309	35	172.515	89	267	12	36		
Ethyl formate	109-94-4	2434	339	26	184.1295			1	3	34	102
Ethyl heptanoate	106-30-9	2437	365	32	172.515	37	111			3	9
Ethyl hexanoate	123-66-0	2439	310	31	172.515			8	24		
Ethyl isobutyrate	97-62-1	2428	288	186	172.515	3	9			1	3
Ethyl isovalerate	108-64-5	2463	442	196	172.515			79	237		
Ethyl laurate	106-33-2	2441	375	37	172.515			156	468	7	21
Ethyl maltol	4940-11-8	3487	692	1481	172.515			97	291	5	15

(*continued on next page*)

Table A (*continued*)

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
Ethyl myristate	124-06-1	2445	385	38	172.515	20	60			6	18
Ethyl nonanoate	123-29-5	2447	388	34	172.515			1	3		
Ethyl octanoate	106-32-1	2449	392	33	172.515	51	153			3	9
Ethyl oleate	111-62-6	2450	633	345	172.515	74	222			5	15
Ethyl palmitate	628-97-7	2451	634	39		8	24				
Ethyl phenylacetate	101-97-3	2452	2156	1009	172.515					2	6
Ethyl propionate	105-37-3	2456	402	28	172.515			3	9	174	522
Ethyl salicylate	118-61-6	2458	432	900	172.515			1	3		
Ethyl *trans*-2, *cis*-4-decadienoate	3025-30-7	3148	10574	1192		1	3				
Ethyl valerate	539-82-2	2462	465	30	172.515			1	3		
Ethyl vanillin	121-32-4	2464	108	893	182.60	28	84			6	18
Eugenol	97-53-0	2467	171	1529	184.1257			1048	3144	1	3
Eugenyl acetate	93-28-7	2469	210	1531	172.515					138	414
Fenugreek absolute	84625-40-1	2485	460		182.20	44	132			17	51
Fenugreek extract	68990-15-8	2484	460		182.10	4	12	266	798		
Furaneol	3658-77-3	3174		1446				1	3	43	129
Galangal (chinese ginger) oleoresin	8024-40-6	2500	29		172.510					3	9
Galbanum oil	8023-91-4	2501	197		172.510			1	3		
Gamma-decalactone	706-14-9	2360	2230	231	172.515	3	9			1	3
Gamma-heptalactone	105-21-5	2539	2253	225	172.515			1	3	1	3
Gamma-hexalactone	695-06-7	2556	2254	223	172.515			1	3	1	3
Gamma-nonalactone	104-61-0	2781	178	229	172.515			1	3	13	39
Gamma-octalactone	104-50-7	2796	2274	226	172.515			1	3	13	39
Gamma-undecalactone	104-67-6	3091	179	233	172.515	4	12			1	3
Gamma-valerolactone	108-29-2	3103	757	220				1	3	1	3
Geraniol	106-24-1	2507	60	1223	182.60	2	6			1	3
Geranium rose oil	8000-46-2							1	3	1	3
Geranyl acetate	105-87-3	2509	201	58	182.60	1	3				
Geranyl isovalerate	109-20-6	2518	448	75	172.515			1	3		
Geranyl phenylacetate	102-22-7	2516	231	1020	172.515	1	3				
Geranyl propionate	105-90-8	2517	409	62	172.515			1	3		
Ginger extract	8002-60-6	2521	489		182.20					13	39
Ginger oil	8007-08-7	2522	489					1	3	1	3
Guaiac wood oil	8016-23-7	2534	220		172.510					1	3
Guaiacol	90-05-1	2532	173	713	172.515			1	3	10	30
Helianthus annuus oil	8001-21-6							1	3		
Heptanoic acid	111-14-8	3348	28	96		1	3			1	3
Hexanal	66-25-1	2557	96	92	172.515			1	3		
Hexanoic acid	142-62-1	2559	9	93	172.515	13	39			3	9
Hexen-2-al	6728-26-3	2560	748	1353	748	3	9			1	3
Hexenyl formate cis-3	33467-73-1	3353		123				1	3		
Hexyl acetate	142-92-7	2565	196	128	172.515	1	3				
Hexyl alcohol	111-27-3	2567	53	91	172.515					1	3
Immortelle absolute	90045-56-0									6	18
Immortelle extract	8023-95-8	2592	-	-	182.20			10	30		
Irone alpha	79-69-6	2597	145	403	172.515			1	3		
Isoamyl acetate	123-92-2	2055	214	43	172.515	54	162			4100	12300
Isoamyl alcohol	123-51-3	2057	51	52	172.515			9	27	110	330
Isoamyl butyrate	106-27-4	2060	282	45	172.515			272	816	31	93
Isoamyl caprylate	2035-99-6	2080	401	47	172.515					1	3
Isoamyl hexanoate	2198-61-0	2075	320	46	172.515	2	6			3	9
Isoamyl isovalerate	659-70-1	2085	458	50	172.515	247	741			4	12
Isoamyl phenylacetate	102-19-2	2081	2161	1014	172.515					1	3
Isoamyl propionate	105-68-0	2082	417	44	172.515			30	90	2	6
Isobutyl alcohol	78-83-1	2179	49	251	172.515	44	132				
Isobutyl phenylacetate	102-13-6	2210	2160	1013	172.515	1	3			1	3
Isobutyraldehyde	78-84-2	2220	92	252	172.515	1	3				
Isobutyric acid	79-31-2	2222	6	253	172.515	1	3				
Isovaleric acid	503-74-2	3102	8	259		8	24			7	21
Jackfruit extract								4	12		
Jasmine absolute	8022-96-6	2600	245		182.20			1	3		
Kola nut extract	68916-19-8	2607	-		182.20					4	12
Labdanum absolute	8016-26-0	2609	134		172.515			7	21	4	12
Lactic acid	598-82-3			930				10	30	213	639
Lactone c-04 gamma	96-48-0	3291	615	219						1	3
Lauric acid	143-07-7	2614	12	111	172.860	1	3			1	3
Lavender oil	8000-28-0	2622	257		182.20			1	3		
Lemon oil	8008-56-8	2625	139		182.20			87	261		
Lemon extract	84929-31-7	2623	139		182.20	1	3			65	195
Lemon verveine	977047-96-3							1	3		
Licorice extract	68916-91-6	2630	218		184.1408	1	3	3844	9610		
Linalool	78-70-6	2635	61	356	182.60	6	18			1	3

Table A (*continued*)

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
Linalyl acetate	115-95-7	2636	203	359	182.60	30	90	1	3		
Linoleic acid	60-33-3		694	332	184.1065	1	3				
L-Lactic acid	79-33-4	2611	4	930	184.1061	10	30	213	639		
L-Lenthol natural	2216-51-5	2665	63	427	172.515			2	6	3997	11991
L-Lenthone	14073-97-3	2267	2035	429	172.515			1	3		
Lovage extract	8016-31-7	2651	261		172.510			2	6	12	36
Mace oil	8007-12-3	2653	296		182.20			40	120	1	3
Maltol	118-71-8	2656	148	1480	172.515			3	9	2	6
Mate absolute	68916-96-1		237		182.20			3	9		
Mate absolute	73296-98-7		237		182.20			1	3	3	9
m-Mimethoxybenzene	151-10-0	2385	189	1249	172.515	1	3			1	3
Menthyl acetate	89-48-5	2668	206	431	172.515	1	3				
Menthyl isovalerate	16409-46-4	2669	450	432	172.515					3	9
Methoxy-3-methylpyrazine (mixture of isomer)	2847-30-5	3183	2266	788		1	3			1	3
Methyl 2-hexenoate	13894-63-8	2709	583		172.515	1	3				
Methyl 2-methylbutyrate	868-57-5	2719	2085	205	172.515	1	3				
Methyl 2-octynoate	111-12-6	2729	481	1357	172.515			1	3		
Methyl 3-methylthiopropionate	13532-18-8	2720	428	472	172.515			1	3		
Methyl acetate	79-20-9	2676	213	125	172.515			1	3	5	15
Methyl benzoate	93-58-3	2683	260	851	172.515			1	3	1	3
Methyl caproate	106-70-7	2708	319	1871	172.515					2	6
Methyl caprylate	111-11-5	2728	398	173	172.515			1	3		
Methyl cinnamate	103-26-4	2698	333	658	172.515	1	3			1	3
Methyl dihydrojasmonate	24851-98-7	3408	10785					1	3		
Methyl heptanoate	106-73-0	2705	368	167	172.515			1	3		
Methyl phenylacetate	101-41-7	2733	2155	1008	172.515			1	3		
Methyl salicylate	119-36-8	2745	433	899						2	6
Methyl sulfide	75-18-3	2746	483	452	172.515	2	6			1	3
Methyl-cyclopentenolone (maple lactone)	80-71-7	2700	758	418	172.515	36	108			6	18
Molasses, blackstrap	8052-35-5		S2							1	3
Myristic acid	544-63-8	2764	16	113	172.860	1	3				
Nerol	106-25-2	2770	2018	1224	172.515	1	3			1	3
Neroli oil	8016-38-4	2771	136		182.20			1	3		
Nonanoic acid	112-05-0	2784	29	102	172.515	15	45			1	3
Nootkatone	4674-50-4	3166	11164	1398	172.515	1	3	71	213	1	3
Oakmoss absolute	9000-50-4	2795	194		172.510	1	3				
Octanoic acid	124-07-2	2799	10	99	184.1025	1	3			1	3
Oleic acid	112-80-1	2815	13	333	172.860	60	180				
Orange oil (distilled)	65996-98-7							1	3		
Orange oil terpenes	68647-72-3	2824								16	48
Orange oil terpenes	68917-57-7	2824	143							84	252
Orange oil, sweet	8008-57-9	2825	143					224	672	1	3
Orange peel sweet oil terpeneless	8028-48-6	2824				124	372			1	3
Origanum oil	8007-11-2	2828	454		182.20			1	3		
Orris root extract	8002-73-1	2830	241		172.515			1	3		
Palmarosa oil	8014-19-5	2831	40		182.20			1	3	1	3
Palmitic acid natural	57-10-3	2832	14	115	172.860	1	3				
Para-cymene	99-87-6	2356	620	1325	172.515	1	3			1	3
Para-methoxybenzaldehyde	123-11-5	2670	103	878	172.515			3	9	8	24
Para-tolyl acetate	140-39-6	3073	226	699	172.515			1	3	1	3
Patchouli oil	8014-09-3	2838	353		172.510			1	3		
Pepper oil, black	8006-82-4	2845	347		182.20			30	90	2	6
Peppermint oil	8006-90-4	2848	282		182.20					3	9
Petitgrain oil	8014-17-3	2854						1	3		
Phenethyl acetate	103-45-7	2857	221	989	172.515	1	3			1	3
Phenethyl alcohol	60-12-8	2858	68	987	172.515	2	6			3	9
Phenethyl butyrate	103-52-6	2861	506	991	172.515					1	3
Phenethyl cinnamate	103-53-7	2863	336	671	172.515	3	9			1	3
Phenethyl isobutyrate	103-48-0	2862	302	992	172.515			1	3	5	15
Phenethyl isovalerate	140-26-1	2871	461	994	172.515	8	24				
Phenethyl tiglate	55719-85-2	2780	2186	997	172.515	1	3			1	3
Phenyl acetaldehyde dimethyl acetal	101-48-4	2876	40	1003	172.515					2	6
Phenylacetaldehyde	122-78-1	2874	116	1002	172.515			1	3	1	3
Phenylacetic acid	103-82-2	2878	672	1007	172.515			10	30	5	15
Pine needle oil	8021-29-2	2905	5		172.510			1	3		
Pine oil, scotch	8023-99-2	2906	341		172.510					1	3
Pineapple extract								4	12		
Piperonal	120-57-0	2911	104	896	182.60			163	489	3	9
p-Mentha-8-thiol-3-one	38462-22-5	3177		561				1	3		

(*continued on next page*)

Table A (*continued*)

Ingredient	CAS No.	FEMA No.	Council of Europe	JECFA	CFR Ref.	Concentration (ppm)					
						Mix A low	Mix A high	Mix B low	Mix B high	Mix C low	Mix C high
Propenylguaethol	94-86-0	2922	170	1264	172.515			1	3		
Propionic acid	79-09-4	2924	3	84	184.1081			1	3	19	57
Propyl acetate	109-60-4	2925	192	126	172.515			1	3		
Prune juice (concentrate)	90082-87-4									586	1758
Pyruvic acid	127-17-3	2970	19	936	172.515					7	21
Rhodinol	6812-78-8	2980	76	1222	172.515			1	3	1	3
Rose absolute	8007-01-0	2988						1	3		
Rum flavour, non-alcoholic	90604-30-1							1	3		
Salicylaldehyde	90-02-8	3004	605	897	172.515	1	3			1	3
Sandalwood oil, yellow	8006-87-9	3005	420		172.510			1	3		
Sandalwood oil, yellow	84787-70-2									1	3
Sodium benzoate	532-32-1	3025			184.1733					3	9
Star anise oil	68952-43-2	2096	238		182.60					13	39
Storax	8046-19-3	3036	265		172.510	1	3			1	3
Styrax benzoin gum	2593-35-2					1	3				
Styrax extract	8024-01-9							1	3	1	3
Sugar: high fructose corn syrup 42%	8029-43-4									14	42
Sugar: invert sugar	8013-17-0					7989	19975				
Sugar: sucrose	57-50-1							269	807	10	30
Tabanon	13215-88-8	4663								4	12
Tagetes oil	8016-84-0	3040	443		172.510					1	3
Tangerine oil	8008-31-9	3041			182.20			1	3		
Tea extract	84650-60-2		277			1	3			14	42
Terpinolene	586-62-9	3046	2115	1331	172.515			1	3	1	3
Terpinyl acetate	8007-35-0	3047	205	368	172.515	2	6				
Terpinyl acetate	80-26-2									2	6
Thymol	89-83-8	3066	174	709	172.515					2	6
Tolu balsam gum	9000-64-0	3070	297		172.510	219	657	2	6		
Trans-2-hexenol	928-95-0							1	3	1	3
Trans-anethole	4180-23-8	2086	183	217	182.60			221	663		
Triacetin	102-76-1	2007		920	184.1901	75	225	9	27		
Triethyl citrate	77-93-0	3083		629	184.1911			480	1440	1	3
Valencene	4630-07-3	3443	11030	1337						2	6
Valeraldehyde	110-62-3	3098	93	89	172.515			1	3	1	3
Valerian root extract	8057-49-6	3100	473		172.510					13	39
Valerian root oil	8008-88-6	3100	473		172.510			1	3		
Valeric acid	109-52-4	3101	7	90	172.515	1	3			1	3
Vanilla extract	8024-06-4	3104	474		182.10			51	153	2	6
Vanilla infusion	8047-24-3					1	3				
Vanillin	121-33-5	3107	107	889	182.60			29	87	87	261
Veratraldehyde	120-14-9	3109	106	877	172.515			1	3	1	3

Remarks: CAS number, numerical identifier assigned by Chemical Abstracts Service; FEMA number, numerical identifier assigned by Flavor and Extract Manufacturers Association, JECFA number, numerical identifier assigned by Joint FAO/WHO Expert Committee on Food Additives; CFR number, numerical identifier assigned by Code of Federal Regulation, USA.

Table B
Mainstream smoke constituent concentrations per cigarette.

Analyte	Unit	2R4F			Control			Mix A low			Mix A high		
		M	SE	N	M	SE	N	M	SE	N	M	SE	N
ISO parameters													
Puff	count	8.08	0.05	4	15.4	0.1	4	16.2	0.1	4	15.4	0.2	4
TPM	mg/cig.	9.62	0.12	4	34.7	0.7	4	34.6	0.2	4	35.0	0.4	4
Tar	mg/cig.	7.83	0.1	4	29.6	0.6	4	29.3	0.2	4	29.7	0.4	4
Nicotine	mg/cig.	0.713	0.01	4	2.13	0.04	4	2.12	0.01	4	2.00	0.03	4
Water	mg/cig.	1.08	0.03	4	3.00	0.11	4	3.17	0.03	4	3.28	0.04	4
Carbon monoxide	mg/cig.	11.1	0.3	4	19.2	0.5	4	18.8	0.2	4	18.1	0.2	4
Aldehydes													
Formaldehyde	μg/cig.	15.8	0.7	5	46.6	1.1	10	47.5	0.7	10	48.2	1.1	10
Acetaldehyde	μg/cig.	498	17	5	716	14	10	741	6	10	698	10	10
Acrolein	μg/cig.	53.2	1.8	5	91.5	1.7	10	95.0	1.1	10	91.6	1.5	10
Propionaldehyde	μg/cig.	44.5	1.3	5	73.9	1.5	10	76.2	1.0	10	73.8	1.0	10
Crotonaldehyde	μg/cig.	10.1	0.4	5	21.4	0.8	10	23.3	0.6	10	22.4	0.5	10
Aliphatic dienes													
1,3-Butadiene	μg/cig.	39.2	1.1	4	82.1	0.7	5	80.1	1.5	5	82.4	3.6	5
Isoprene	μg/cig.	391	10	4	533	13	5	535	15	5	523	13	5
Acid derivatives													
Acetamide	μg/cig.	3.94	0.06	4	10.9	0.3	5	9.93	0.12	5	10.9	0.3	5

Table B (*continued*)

Analyte	Unit	2R4F			Control			Mix A low			Mix A high		
		M	SE	N	M	SE	N	M	SE	N	M	SE	N
Acrylamide	μg/cig.	1.56	0.03	4	4.32	0.18	5	3.81	0.15	5	4.26	0.26	5
Acrylonitrile	μg/cig.	28.0	0.2	4	52.0	0.9	5	51.2	1.2	5	51.8	2.0	5
Nitro compounds													
2-Nitropropane	ng/cig.	16.8	0.4	4	19.7	0.6	4	20.9	0.7	4	19.8	0.7	4
Aromatic amines													
o-Toluidine	ng/cig.	49.4	0.5	8	47.7	0.5	8	47.2	0.8	8	47.3	0.5	8
o-Anisidine	ng/cig.	2.05	0.02	8	1.45	0.05	8	1.42	0.06	7	1.41	0.02	8
2-Naphthylamine	ng/cig.	5.57	0.1	8	4.99	0.15	8	5.19	0.18	8	4.97	0.05	8
4-Aminobiphenyl	ng/cig.	1.04	0.03	8	0.844	0.03	8	0.927	0.047	7	1.01	0.02	7
Halogen compounds													
Vinyl chloride	ng/cig.	34.9	0.8	4 5	65.9	1.7	5	76.1	1.6	5	66.9	2.2	5
Inorganic compounds													
Nitrogen oxides	μg/cig.	227	1	4 5	151	3	5	155	4	5	148	2	5
Hydrogen cyanide	μg/cig.	81.6	3.2	4 4	194	5	4	187	8	4	194	4	4
Monocyclic aromatic hydrocarbons													
Benzene	μg/cig.	45.6	1.6	4	87.6	1.7	5	87.8	0.9	5	92	2.2	5
Toluene	μg/cig.	78.5	1	4	129	3	5	127	1	5	135	3	5
Styrene	μg/cig.	6.16	0.16	4	13.7	0.2	5	14.2	0.1	5	15.9	0.4	5
Volatile N-nitrosamines													
NDMA	ng/cig.	<5.00		4	<5.00		4	<5.00		4	<5.00		4
NMEA	ng/cig.	<10.0		4	<10.0		4	<10.0		4	<10.0		4
NDEA	ng/cig.	<7.00		4	<7.00		4	<7.00		4	<7.00		4
NPRA	ng/cig.	<11.0		4	<11.0		4	<11.0		4	<11.0		4
NBUA	ng/cig.	<9.00		4	<9.00		4	<9.00		4	<9.00		4
NPY	ng/cig.	<7.00		4	<7.00		4	<7.00		4	<7.00		4
NPI	ng/cig.	<8.00		4	<8.00		4	<8.00		4	<8.00		4
Tobacco-specific N-nitrosamines													
NNN	ng/cig.	113	8	4	36.7	3.5	10	34.6	2.4	10	29.2	0.9	10
NNK	ng/cig.	105	7	4	17.9	1.6	10	17.6	1.0	10	14.7	0.6	10
Phenols													
Phenol	μg/cig.	8.13	0.2	4	48.8	0.6	5	45.6	0.5	5	45.5	0.8	5
Catechol	μg/cig.	39.3	1.2	4	131	1	5	127	1	5	124	1	5
Hydroquinone	μg/cig.	29.3	0.9	4	80.3	0.9	5	79.1	1.1	5	76.5	1	5
Eugenol	mg/cig.	0		4	5.57	0.1	5	5.29	0.21	5	5.23	0.05	5
Polycyclic aromatic hydrocarbons													
Pyrene	ng/cig.	37.8	0.4	4	141	3	5	154	3	5	145	4	5
Benz[a]anthracene	ng/cig.	12.5	0.1	4	45.6	1.0	5	48.7	0.9	5	48.0	1.0	5
Benzo[b]fluoranthene	ng/cig.	5.05	0.05	4	17.5	0.4	5	18.6	0.3	5	18.1	0.4	5
Benzo[j]fluoranthene	ng/cig.	3.18	0.06	4	12.0	0.2	5	12.6	0.3	5	12.3	0.4	5
Benzo[k]fluoranthene	ng/cig.	1.97	0.04	4	7.26	0.21	5	7.78	0.18	5	7.29	0.24	5
Benzo[a]pyrene	ng/cig.	6.82	0.07	4	25.1	0.5	5	26.8	0.4	5	26.3	0.5	5
Dibenz[a,h]anthracene	ng/cig.	<0.970		4	1.53	0.05	5	1.48	0.05	5	1.64	0.05	5
Dibenzo[a,e]pyrene	ng/cig.	0.19	0.007	4	0.674	0.02	5	0.7	0.025	5	0.654	0.012	5
Dibenzo[a,h]pyrene	ng/cig.	<0.230		4	0.78	0.02	5	0.836	0.032	5	0.78	0.027	5
Dibenzo[a,i]pyrene	ng/cig.	<0.220		4	0.562	0.01	5	0.606	0.021	5	0.574	0.022	5
Dibenzo[a,l]pyrene	ng/cig.	<0.190		4	<0.190		5	<0.190		5	<0.190		5
Indeno(1,2,3-cd)pyrene	ng/cig.	2.89	0.06	4	10.6	0.2	5	11.1	0.2	5	10.8	0.3	5
5-Methylchrysene	ng/cig.	<0.400		4	<0.400		5	<0.400		5	<0.400		5
Metals													
Arsenic	ng/cig.	2.79	0.09	4	<4.31		4	<4.31		4	<4.31		4
Cadmium	ng/cig.	38.8	1.1	4	102	2	4	103	3	4	98.2	1.5	4
Chromium	ng/cig.	<1.60		4	<4.92		4	<4.92		4	6.06		4
Nickel	ng/cig.	<2.10		4	<6.46		4	<6.46		4	<6.46		4
Lead	ng/cig.	11.2	0.4	4	17.3	0.3	4	17.8	0.7	4	19.5	0.4	4

Parameter	Unit	Mix B low			Mix B high			Mix C low			Mix C high		
		M	SE	N	M	SE	N	M	SE	N	M	SE	N
ISO parameters													
Puff	count	15.4	0.3	4	14.6	0.1	4	15.7	0.2	4	15.5	0.2	4
TPM	mg/cig.	34.4	0.4	4	34.7	0.5	4	35.9	0.7	4	36.6	0.9	4
Tar	mg/cig.	29.5	0.4	4	29.9	0.3	4	30.6	0.7	4	31.4	0.9	4
Nicotine	mg/cig.	2.04	0.01	4	1.98	0.02	4	2.03	0.02	4	2.06	0.02	4
Water	mg/cig.	2.95	0.05	4	2.84	0.14	4	3.27	0.09	4	3.14	0.09	4
Carbon monoxide	mg/cig.	19.7	0.2	4	18.5	0.5	4	18.5	0.2	4	18.7	0.3	4
Aldehydes													
Formaldehyde	μg/cig.	46.3	1.3	10	50.3	0.5	10	45.4	1.3	10	46.5	1.4	10
Acetaldehyde	μg/cig.	747	14	10	721	11	10	710	18	10	716	18	10
Acrolein	μg/cig.	99	2	10	94.2	1.3	10	90.9	2.5	10	92.1	2.5	10

(*continued on next page*)

Table B (*continued*)

Parameter	Unit	Mix B low			Mix B high			Mix C low			Mix C high		
		M	SE	N	M	SE	N	M	SE	N	M	SE	N
Propionaldehyde	µg/cig.	79.6	1.1	10	75.2	1.2	10	73.1	1.7	10	76.7	2	10
Crotonaldehyde	µg/cig.	22.8	0.9	10	20.8	0.8	10	22.3	0.7	10	22.2	0.5	10
Aliphatic dienes													
1,3-Butadiene	µg/cig.	82.2	3.2	5	75.5	1.5	5	89.7	1.4	5	83.4	2.1	5
Isoprene	µg/cig.	543	10	5	502	8	5	554	15	5	523	8	5
Acid derivatives													
Acetamide	µg/cig.	11.6	0.3	5	10.2	0.2	5	12.1	0.2	5	12	0.1	5
Acrylamide	µg/cig.	4.08	0.25	5	4.32	0.14	5	4.15	0.24	5	4.08	0.2	5
Acrylonitrile	µg/cig.	52.8	1.6	5	48.2	1.3	5	54.9	1.7	5	53	0.6	5
Nitro compounds													
2-Nitropropane	ng/cig.	21	0.9	4	21.7	1.2	4	19.6	0.5	4	20.9	1.2	4
Aromatic amines													
o-Toluidine	ng/cig.	48.8	0.6	8	46.6	0.5	8	47.5	0.8	8	48.4	0.8	8
o-Anisidine	ng/cig.	1.51	0.05	8	1.45	0.04	8	1.43	0.03	8	1.48	0.05	8
2-Naphthylamine	ng/cig.	5.41	0.12	8	5.37	0.07	8	4.9	0.15	8	5.21	0.21	8
4-Aminobiphenyl	ng/cig.	0.945	0.027	8	1	0.02	7	0.849	0.027	8	0.875	0.035	8
Halogen compounds													
Vinyl chloride	ng/cig.	67.8	2.6	5	64.1	1.9	5	77.3	2.4	5	77.4	2.8	5
Inorganic compounds													
Nitrogen oxides	µg/cig.	149	1	5	146	3	5	149	3	5	149	3	5
Hydrogen cyanide	µg/cig.	207	7	4	196	9	4	199	5	4	194	5	4
Monocyclic aromatic hydrocarbons													
Benzene	µg/cig.	88.5	1.7	5	81.8	1.9	5	92.9	1.4	5	85.9	0.9	5
Toluene	µg/cig.	128	3	5	115	2	5	129	2	5	115	1	5
Styrene	µg/cig.	13.7	0.4	5	12.3	0.3	5	14.2	0.3	5	13.2	0.1	5
Volatile N-nitrosamines													
NDMA	ng/cig.	<5.00		4	<5.00		4	<5.00		3	<5.00		4
NMEA	ng/cig.	<10.0		4	<10.0		4	<10.0		3	<10.0		4
NDEA	ng/cig.	<7.00		4	<7.00		4	<7.00		3	<7.00		4
NPRA	ng/cig.	<11.0		4	<11.0		4	<11.0		3	<11.0		4
NBUA	ng/cig.	<9.00		4	<9.00		4	<9.00		3	<9.00		4
NPY	ng/cig.	<7.00		4	<7.00		4	<7.00		3	<7.00		4
NPI	ng/cig.	<8.00		4	<8.00		4	<8.00		3	<8.00		4
Tobacco-specific N-nitrosamines													
NNN	ng/cig.	41.1	2.2	10	41.7	1.9	10	42	1.8	10	40.8	2.3	10
NNK	ng/cig.	22.1	2.3	10	22.2	2.7	10	20.2	0.7	10	23.3	3.7	10
Phenols													
Phenol	µg/cig.	46.8	0.6	5	46.7	1	5	49.1	0.6	5	47.6	0.5	5
Catechol	µg/cig.	127	1	5	126	2	5	128	1	5	126	1	5
Hydroquinone	µg/cig.	77.8	1.3	5	78	1.5	5	77.6	0.4	5	79.2	0.7	5
Eugenol	mg/cig.	5.31	0.12	5	6.24	0.05	5	5.67	0.06	5	5.48	0.14	5
Polycyclic aromatic hydrocarbons													
Pyrene	ng/cig.	144	2	5	148	3	5	143	2	5	148	4	5
Benz[a]anthracene	ng/cig.	45.7	0.9	5	46.9	0.9	5	45.4	0.5	5	47.8	1.2	5
Benzo[b]fluoranthene	ng/cig.	17.6	0.3	5	18.1	0.3	5	17.3	0.1	5	18	0.4	5
Benzo[j]fluoranthene	ng/cig.	12.3	0.3	5	12	0.3	5	12.1	0.2	5	12.2	0.2	5
Benzo[k]fluoranthene	ng/cig.	7.73	0.18	5	7.48	0.22	5	7.24	0.2	5	7.41	0.22	5
Benzo[a]pyrene	ng/cig.	25.4	0.3	5	25.9	0.4	5	24.9	0.3	5	26.5	0.6	5
Dibenz[a,h]anthracene	ng/cig.	1.46	0.06	5	1.55	0.04	5	1.54	0.07	5	1.49	0.04	5
Dibenzo[a,e]pyrene	ng/cig.	0.678	0.018	5	0.692	0.018	5	0.682	0.024	5	0.67	0.014	5
Dibenzo[a,h]pyrene	ng/cig.	0.778	0.02	5	0.8	0.023	5	0.766	0.016	5	0.818	0.022	5
Dibenzo[a,i]pyrene	ng/cig.	0.584	0.009	5	0.594	0.007	5	0.564	0.012	5	0.542	0.02	5
Dibenzo[a,l]pyrene	ng/cig.	<0.190		5	<0.190		5	<0.190		5	<0.190		5
Indeno(1,2,3-cd)pyrene	ng/cig.	10.5	0.2	5	10.7	0.2	5	10.4	0.2	5	10.9	0.3	5
5-Methylchrysene	ng/cig.	<0.400		5	<0.400		5	<0.400		5	<0.400		5
Metals													
Arsenic	ng/cig.	<4.31		4	<4.31		4	<4.31		4	<4.31		4
Cadmium	ng/cig.	98.6	3.9	4	93.9	2.1	4	91.8	1	4	95	4.1	4
Chromium	ng/cig.	<4.92		4	6.15		4	5.28		4	<4.92		4
Nickel	ng/cig.	<6.46		4	<6.46		4	<6.46		4	<6.46		4
Lead	ng/cig.	17.9	0.5	4	17.1	0.2	4	16.2	0.3	4	17.3	0.3	4

Remarks: N, number of measurements; M, arithmetic mean, SE, standard error.

Table C
In vitro cytotoxic, mutagenic, and genotoxic activities on a per cigarette basis.

Test system, Test material		Cigarette type 2R4F a	Control a	Mix A low	Mix A high	Mix B low	Mix B high	2R4F b	Control b	Mix C low	Mix C high
NRU assay, 1/EC50 (ml/cig.)											
TPM		112 ± 5	391 ± 5	380 ± 20	368 ± 11	379 ± 8	376 ± 8	110 ± 5	370 ± 14	356 ± 12	366 ± 6
GVP		83 ± 1	122 ± 1	117 ± 3	111 ± 5	122 ± 2	116 ± 1	81 ± 6	112 ± 2	105 ± 4	111 ± 7
Ames assay, slope (revertants/cig.)											
TPM											
+S9	TA98	29069 ± 814	42716 ± 2292	41964 ± 1722	34965 ± 2491	39463 ± 2581	40316 ± 2246	–	–	46131 ± 2006	44798 ± 2125
	TA100	13747 ± 800	24998 ± 2224	29696 ± 3054	25451 ± 3027	27330 ± 1675	26178 ± 2161	–	–	26300 ± 1993	29253 ± 3046
	TA102	29 ± 134	−202 ± 697	−109 ± 762	237 ± 782	−1067 ± 702	−1123 ± 690	–	–	−492 ± 715	−208 ± 695
	TA1535	18 ± 9	98 ± 40	12 ± 57	118 ± 37	65 ± 56	21 ± 59	–	–	36 ± 55	154 ± 63
	TA1537	4778 ± 423	6299 ± 921	6413 ± 929	7405 ± 798	7256 ± 1181	6701 ± 790	–	–	6281 ± 771	7315 ± 923
−S9	TA98	66 ± 27	139 ± 89	109 ± 93	−4 ± 91	251 ± 87	163 ± 68	–	–	404 ± 121	290 ± 91
	TA100	849 ± 177	2368 ± 644	4126 ± 620	3134 ± 660	3085 ± 587	2027 ± 575	–	–	1676 ± 679	3167 ± 636
	TA102	208 ± 139	439 ± 382	−89 ± 532	−298 ± 591	−54 ± 705	−421 ± 655	–	–	612 ± 440	−322 ± 380
	TA1535	−2 ± 15	23 ± 51	35 ± 53	−33 ± 53	−53 ± 55	−144 ± 76	–	–	−65 ± 63	86 ± 77
	TA1537	130 ± 20	239 ± 50	244 ± 69	279 ± 71	85 ± 75	187 ± 72	–	–	287 ± 69	170 ± 64
MLA assay, 1/C3B (ml/cig.)											
TPM											
+S9		57 (72, 49)	393 (505, 320)	362 (508, 249)	338 (353,313)	367 (444, 305)	385 (457, 318)	57 (54, 58)	425 (453, 248)	376 (427, 338)	401 (457, 358)
−S9		315 (299, 332)	1062 (1037, 1152)	1022 (1068, 1022)	1214 (1228, 1206)	1086 (1035, 1332)	1025 (987, 992)	254 (283, 234)	871 (1020, 782)	827 (981, 735)	943 (969, 920)

Remarks: NRU, Neutral Red Uptake Assay; MLA, Mouse Lymphoma Assay; data give means and standard deviations (in case of the MLA, results of the two determinations).

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